Dielectric Behavior of Biological Cells and Membranes (Commemoration Issue Dedicated to Professor Tetsuya HANAI On the Occasion of His Retirement)

AUTHOR(S):

Schwan, Herman P.; Takashima, Shiro

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Herman P. SCHWAN* and Shiro TAKASHIMA*

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1. INTRODUCTION

The electrical properties of biological cells and tissues are unusual in many ways: Their relative dielectric constants often approach extremely high values as the frequency decreases below 1 KHz, reaching values in excess of 10^6. The theory of polar molecules which has been the most important mathematical tool to explain the dipole moment of a variety of organic compounds is unable to account for this extraordinary dielectric behavior. Furthermore, the dielectric constant and conductivity of these materials decrease in 3 or 4 discrete steps from an extremely large value to approximately 4 between 1Hz and 100GHz. This observation indicates the existence of several polarization mechanisms in biological matters. We will discuss the origin of this rather anomalous but unique dielectric behavior of biological materials and the mathematical theories which are needed to explain these polarizations.

Interest in the response of biological materials to electricity developed some 200 years ago with the pioneering work of Galvani and Volta. In 1848 du Bois Reymond published his two volume text "Untersuchungen ueber die tierische Elektrizitaet", which gained wide distribution. Others of the Berlin goup include his teacher Mueller and Helmholtz. Helmholtz, combined expertise, to an unusual degree, in physiology and anatomy with physics. During the 1870's important work was done by Herman (1871), first in Zurich and then in Koenigsberg. He determined the resistance anisotropy-ratio of muscle and nervous tissues at DC. He also studied excitability and his results were found to be consistent with the assumption that cells have an envelope surrounding the interior. The envelope, and early concept of a membrane, was thought to contain a "polarization" element which was considered later again by K.S.Cole (1968). It became also clear at the time that excitability threshold current values are much lower if applied in the direction of the fibers instead of across it. Limited instrumentation was applied with great ingenuity at the time.

The early electrophysiology work included: a) study of the electrical conductance of blood and tissues and b) study of excitability and contractility, later recognized to be caused by the nonlinear properties of membranes above certain threshold values. First determinations of the electrical properties of blood were carried out shortly after the introduction of the Wheatstone bridge and the precision conductivity determinations of electrolytes by Kohlrausch. Hoeber (1910,1912) became well known for his

* Herman P. Schwan, Shiro Takashima: Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104-6392, U.S.A.

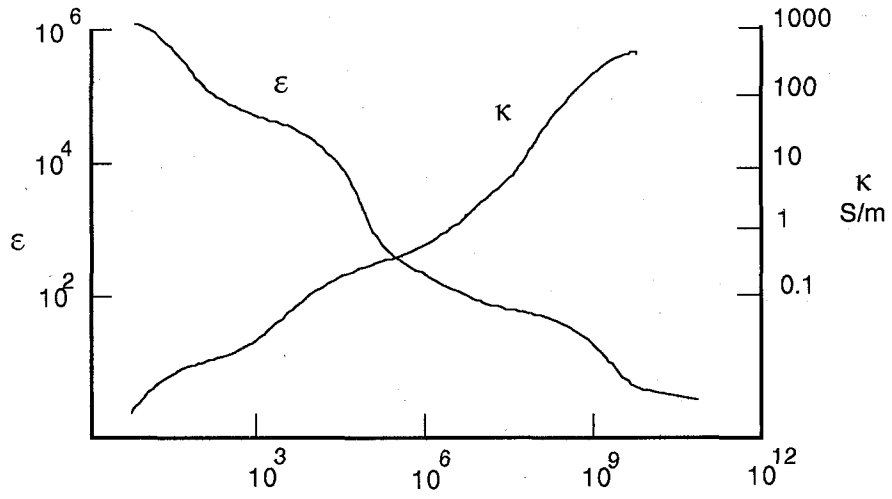

Fig. 1. Stepwise dispersions of biological materials. α-dispersion is due to ionic processes, β-dispersion is due to charging up of the membrane or orientation of permanent dipoles. γ-dispersion is due to the orientational relaxation of water.

early work on red cells. He had compared the conductivities of erythrocytes at low and high frequencies and deduced from the difference the existence of a cell membrane. This was the first work to do so from purely electrical data and done only a few years after Bernstein formulated the membrane hypothesis.

Interest in the electrical properties of cells and tissues increased rapidly during the period separating the two world wars. H. Fricke, K. S. Cole, H. J. Curtis and H. Daenzer were the first to apply rigorous potential theory based on maxwell's early work (Maxwell, 1873) to cell suspensions and advanced instrumentation was applied to collect data spanning an ever increasing frequency range extending from some KHz to about 100 KHz. This work resulted in an explanation of the dispersive behavior in the RF-range (termed β-dispersion by Schwan, 1957). A detailed survey of the earlier work was given by Rajewsky (1938) and later in part I of K. S. Cole's text (Cole, 1968). After world war II the frequency range was extended to include data well below the KHz range, and upwards into the microwave frequency range. Additional and partially unexpected dispersion phenomena were discovered (α, δ and γ, Schwan, 1957 see Fig. 1). Extension into the nonlinear range led to important contributions to membrane biophysics and identification of mechanism responsible for excitability (Cole 1968).

2. ELEMENTS OF DIELECTRIC SPECTROSCOPY

The linear electrical properties of matter are defined by two important parameters such as dielectric constant ε and conductivity $\varkappa$. ε and $\varkappa$ often change with frequency. In the simplest case where dielectric relaxation is characterized by only one time constant, such frequency dependence can be expressed by the equations

$$\varepsilon^* = \varepsilon_\infty + \frac{\varepsilon_0 - \varepsilon_\infty}{1+j\omega\tau} - \frac{j\kappa_0}{\omega} \tag{1}$$

$$K = \kappa_0 + \frac{\kappa_0 - \kappa_\infty}{1+j\omega\tau} + j\omega\varepsilon_\infty \tag{2}$$

The subscripts o and ∞ indicate limit values at low and high frequencies and the third terms $j\omega\varepsilon_\infty$ and $-j\kappa_0/\omega$ have been added to include low and high frequency limits of κ and ε respectively caused by other mechanisms. ε and κ change with frequency from one limit value to another, i. e. are dispersive. The "relaxation" mechanism responsible for this dispersive behavior is characterized by the time constant τ and the dielectric increment $\varepsilon_0 - \varepsilon_\infty$. The frequency dependency stated in eq. (1) corresponds to an exponential time response of dielectric displacement D to a sudden change in field strength and that in eq. (2) to one of current density j. Equations (1) and (2) are interrelated through

$$\varepsilon_0 - \varepsilon_\infty = (\kappa - \kappa_0)\tau \tag{3}$$

If the time response is not a simple exponential function, the behavior may be characterized by a sum or integral of exponential responses. For a discrete number of relaxation processes, eq. (1) can be rewritten as

$$\varepsilon(Re) = \varepsilon_\infty + \Sigma \frac{\Delta\varepsilon_j}{1+(\omega\tau_j)^2} \tag{4}$$

A more generalized equation has been formulated for continuous distribution of relaxation times as shown below.

$$\varepsilon(Re) = \varepsilon_\infty + (\varepsilon_0 - \varepsilon_\infty) \int_0^\infty \frac{G(\tau)d\tau}{1+(\omega\tau)^2} \tag{5}$$

where $G(\tau)$ is a distribution function. A common distribution of such reponses is the logarithmic symmetrical Cole-Cole distribution of time constants. It yields the simple extensions of eqs. (1) and (2)

$$\varepsilon^* = \varepsilon_\infty + \frac{\varepsilon_0 - \varepsilon_\infty}{1+(j\omega\tau)^\alpha} - \frac{j\kappa_0}{\omega} \tag{6}$$

$$K = \kappa_0 + \frac{\kappa_\infty - \kappa_0}{1+(j\omega\tau)^\alpha} + j\omega\varepsilon_\infty \tag{7}$$

Equation (6) reduces to equation (5) if the distribution function $G(\tau)$ is assumed to be

$$G(s) = \frac{\sin n\pi}{2\pi(1-\cos n\pi)} \tag{8}$$

where $n = 1-\alpha$ and $s = \tau/\tau_0$.

Equation (6) yields circles in the dielectric plane (plot of ε'' vs ε') if $\kappa_0 = 0$ and eq. (7) circles in the admittance plane ($\omega\varepsilon'$ vs. κ) if $\varepsilon_\infty = 0$. The centers of the circles are

below the real axis if $\alpha < 1$. They move on it as α approaches 1. Plots in dielectric and admittance planes are useful in order to determine limit values at low and high frequencies. For further details see Cole and Cole (1941), Boettcher (1952) and Froehlich (1949).

3. RELAXATION MECHANISM

Relaxation mechanism responsible for dispersive behavior of cells may be caused either by permanent or field induced dipoles.

3.1 Maxwell-Wagner Effect

A strong contribution to dielectric relaxation in biological cell suspensions and tissues is caused by the charging of interfaces separating different cellular compartments. Since $D=\varepsilon E$ and $J=\kappa E$ (D dielectric displacement, J current density and E electrical field strength) D/J is equal to ε/κ. In a system, free of any electrical charge, D is continuous across any given interface. Hence the current density component normal to the interface will vary unless the ratio ε/κ is the same on both sides of the interface. Thus charges will accumulate at the interface with time until J becomes continuous. Several relevant simple models were first treated by Wagner (1914, 1924), who extended DC calculations by Maxwell (1873) to heterogeneous systems exposed to alternating fields. These include an arrangement of two slabs of material in series and suspensions of spherical particles. The admittivity K of a suspension of spherical particles is given by

$$\frac{K-K_a}{K+2K_a} = p\,\frac{K_p-K_a}{K_p+2K_a} \tag{9}$$

Here K_a and K_p indicate the complex admittivity of external medium and particle, p is the particle volume fraction. This equation can be transformed into eq. (1) with the parameters (Pauly and Schwan, 1959)

$$\tau=\frac{(1-p)\varepsilon_i+(2+p)\varepsilon_a}{(1-p)\kappa_i+(2+p)\kappa_a} \tag{10}$$

$$\varepsilon_0-\varepsilon_\infty=\frac{9p(1-p)(\varepsilon_a\kappa_i-\varepsilon_i\kappa_a)^2}{[(1-p)\varepsilon_i+(2+p)\varepsilon_a][(1-p)\kappa_i+(2+p)\kappa_a]^2} \tag{11}$$

where ε_i and κ_i are the permittivity and conductivity of cell interior. The dielectric increment $\varepsilon_0-\varepsilon_\infty$ is usually of the order of a few dielectric units relative to free space, i.e. of small magnitude.

Maxwell's equation, in principle, was derived with an assumption that the number of particles in suspension is very low and, consequently, the superposition theorem of potentials can be used. However, we often have to deal with cases which do not necessarily satisfy this condition. Hanai's equation which is a generalization of Bruggeman's theory (1935), circumvent this difficulty and yields a good agreement with experimental results for particle suspensions with moderate and even high concentra-

tions.

Hanai's theory was, unlike Maxwell's equation, derived without the assumption of dilute particle suspension. Therefore, this equation, in principle, is applicable for condensed systems as well as for dilute suspensions.

$$\frac{\varepsilon^* - \varepsilon_2^*}{\varepsilon_1^* - \varepsilon_2^*} \left(\frac{\varepsilon_1^*}{\varepsilon_2^*}\right)^{1/3} = 1 - P \tag{12}$$

where ε^*, ε_1^* and ε_2^* are the complex permittivities of suspension, external medium and particles ($\varepsilon^* = \varepsilon - j\kappa/\omega$). P is volume fraction. Although this equation cannot be solved analytically, it can be numerically solved using a computer.

We consider next a spherical model for a biological cell, assuming a homogeneous interior, the cytoplasm, surrounded by a membrane. In this case Maxwell's "equivalent homogeneous" particle admittivity equation

$$\frac{K_p - K_m}{K_p + 2K_m} = \frac{R^3}{(R+d)^3} \frac{K_i - K_m}{K_i + 2K_m} \tag{13}$$

can be used to reduce the shell surrounded particle to a homogeneous one (K_p is the equivalent particle admittivity, R core radius, d membrane thickness, i and m indicate cell interior and membrane). Extension of Maxwell's DC-equation to the complex case is justified since all boundary conditions such as continuity of potentials and current density normal to the interfaces remain valid.

Combination of eqs. (9) and (13) gives the electrical properties of a suspension of spherical biological cells with membranes surrounding the internal cytoplasm. The result is given by the sum of two relaxation equations of the type stated in equation (1). For $d \ll R$ and parameters typical of biological cells, the two time constants differ by a factor near 100. Then the following excellent approximations for the parameters result for the low frequency dispersion (Pauly and Schwan, 1959):

$$\tau = RC_m \frac{(2+p)\rho_i + (1-p)\rho_a}{2 + p + RG_m[(2+p)\rho_i + (1-p)\rho_a]} \tag{14}$$

$$\varepsilon_0 - \varepsilon_\infty = \frac{9p}{(2+p)^2} \frac{RC_m}{[1 + RG_m \{\rho_i + \rho_a(1-p)/(2+p)\}]^2} \tag{15}$$

$C_m = \varepsilon_m/d$ and $G_m = \kappa_m/d$ are membrane capacitance and conductance per unit area, $\rho_i = 1/\kappa_i$ and $\rho_a = 1/\kappa_a$ resistivities of internal and external fluids and p volume concentration. The parameters for the high frequency dispersion are identical with eqs. (10) and (11).

Maxwell's derivations of eqs. (9) and (13) assume that counterions form an infinitesimally thin layer near the surface, i. e., there are no space charges except at the surface. Thus Laplace's equation was assumed as a valid representation of the electrical potentials in all phases. This is not quite correct for charged particles suspended in aqueous media and Poisson's equation ought to be solved. However the error has been demonstrated to be small for cellular dimensions which are large compared to the Debye screening length (Garcia *et al*, 1985).

Table 1. Maxwell-Wagner β-dispersion parameters are given for two cell sizes. The following typical cell properties are used; $C_m=1\ \mu F/cm^2$, $r_i=120$ Ohm-cm, $r_a=60$ Ohm•cm, $p=0.3$. The effect of the membrane conductance has been neglected since usually small. The data are theoretically predicted (eq.3, 9-13). The low frequency β_1 dielectric increment (relative to free space) is many orders of magnitude larger than the high frequency β_2 dispersion. The conductivity increments (in mS/cm) differ by a factor near 100.

β-dispersion	$R(\mu m)$	f_c(MHz)	$\varepsilon_0-\varepsilon_\infty$	$\kappa_\infty-\kappa_0$
1	10μm	1.15	5760	3.68
	1	11.5	576	3.68
2	10	370	0.20	0.04
	1	370	0.20	0.04

In Table 1 are listed values for the case of typical cell suspensions. The data demonstrate: 1. The magnitude of low frequency dispersion is very large, resulting in ε-values above 10^3. Thus, the Maxwell-Wagner mechanism predicts a dispersion of a large magnitude at frequencies between 0.1 and 10 MHz. 2. The high frequency dispersion is independent of C_m and G_m. It is, therefore, simply that of a suspension of core particles without a membrane and its magnitude is small. Maxwell-Wagner dispersion effects are to be expected if the composition of matter is heterogeneous with interfaces separating various phases. The accumulation of charges of opposite sign on both sides of the interfaces create large induced dipole moments. Thus the noted dispersion effects are reflective of frequency dependent induced dipoles.

3.2 Counterion relaxation

Another induced dipole relaxation is caused by the tangential movement of counter ions along the surface of particles. A particle carrying fixed charges is surrounded by an atmosphere of counterions. Application of an electrical field results in a displacement of this atmosphere creating thereby an induced dipole moment. Colloidal suspensions displaying dispersions characterized by very large $\varepsilon_0-\varepsilon_\infty$ increments and low characteristic frequencies were first reported by us (Schwan 1957, Schwan *et al,* 1962). Figure 2 presents some typical data. The time constant τ of this strong effect was found to be proportional to R^2.

Schwarz (1962) developed a first model for counterion relaxation, believed to be responsible for this dispersion. His equations for time constant τ and dielectric increment are (e_0, u charge and mobility of counterion; R particle radius, p particle volume concentration, T absolute temperature, σ_0 counterion charge density and k Boltzmann constant)

$$\tau=\frac{R^2}{2ukT} \tag{16}$$

$$\varepsilon_0-\varepsilon_\infty=\frac{9p}{(2+p)^2}\frac{e_0^2R\sigma_0}{\varepsilon_v kT} \tag{17}$$

Schwarz's theory was generalized to apply to prolate ellipsoids and further modified for long rod-like molecules (Takashima, 1967, 1989)

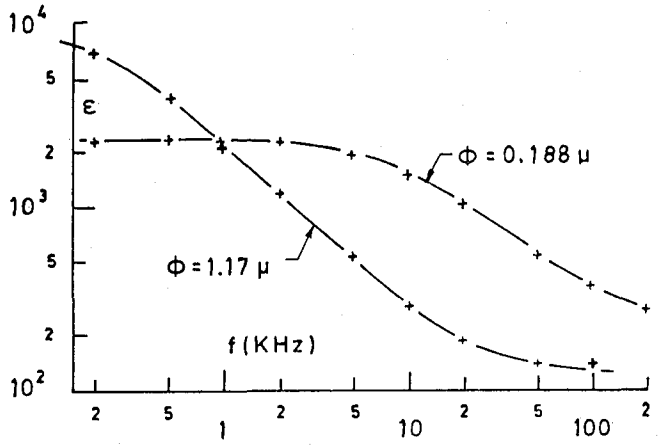

Fig. 2. Dielectric dispersion of poly-styrene particles suspended in aqueous media. Numbers for each curve indicate the radius of particles in μm.

$$\varepsilon^* = \frac{e_0^2 \sigma_0 a^2}{b\varepsilon_v kT} \frac{9p}{(1+p)^2} \frac{1}{1+j\omega\tau} \tag{18}$$

Here, a and b are major and minor axes of very long rods. Other counterion theories have been developed for rod like polyions and these theories are discussed in Part II. Recently, more theories have been formulated (Chew, 1984, Fixman, 1983, Grosse and Foster 1987). There is no further doubt that counterion movement about micron-sized particles creates induced dipoles which result in very high permittivity values at low frequencies. Since biological cells carry many fixed charges, counterion movement does contribute to their dispersive behavior observed at low frequencies.

3.3 Admittance of Biological Membranes

The electrical properties of biological membranes are traditionally characterized by membrane capacitance per unit area C_m and membrane conductance G_m. Typical membrane capacitance values are about $1\mu F/cm^2$ and appear to be frequency independent (Asami *et al.* 1990) between 1Hz and 1KHz. This value corresponds to a lipid film of about 40A with a dielectric constant about 4. Membrane conductanes are more variable and range from 0.1 ms/cm^2 of passive membranes to a much higher value for excitable membranes. Those high conductance values are thought to be caused by opening of the membrane pores or channels.

Membrane admittances at low frequencies change with the voltage and frequency of applied fields. These admittances are mostly due to time dependent ionic currents and are not due to the relaxation of membrane components such as channel proteins. These phenomenological admittances can be explained by the linearized and Laplace transformed Hodgkin-Huxley equation. These frequency domain analyses of time and voltage dependent ionic currents are summarized in the series of articles by Fishman *et al.* (1977, 1985).

4. EXPERIMENTAL DATA

Figure 1 displays the dielectric behavior typical for many tissues and cell suspensions. Three relaxation regions of large magnitude are apparent at low, medium and very high frequencies identified as α, β and γ (Schwan, 1983). There is evidence for a small and broad additional relaxation effect called δ-dispersion for protein solutions between 100 and 1000MHz. Clearly, magnitude and time constants of observed dispersions vary considerably. We consider now the various components of cell suspensions and tissues and their individual dielectric properties.

4.1 Cells

Cells are surrounded by membranes, which separate the medium surrounding the cell from the cytoplasm, i. e., interior phase. There exists an immense variety of biological cells. Prokaryotes are primitive cells which emerged much earlier than the highly differentiated eukariotes. The cytoplasm of the more highly developed eukaryotes contains many smaller cell-like structures including the cell nucleus, mitochondria, lysosomes, golgi apparatus, ribosomes and endoplasmic reticulum, each with a specialized function. Nucleus and other "subcellular organelles" such as mitochondria are surrounded by their own membranes. Some membrane systems interconnect to each other. The cytoplasm contains many proteins. Part of them are arranged in the cytoskeleton, a web of string-like structures which appear to provide some structual rigidity to the cell. It is linked to a similar membrane structure which connects part of the membrane proteins, while others have been shown to move under the influence of electrical fields. The glycocalyx, a loose brushlike structure fixed to the outer surface of the membrane, consists of glycoproteins and sugars. It is highly permeable and rich in fixed charges. Its thickness is about 100A, i. e., comparable to or larger than the Debye screening length for the medium outside the cell.

The electrical properties of cell suspensions have been studied by many investigators particularly Fricke, Cole, Schwan, Carstensen and Hanai and their collegues as reviewed by Cole (1968) and Foster and Schwan (1989). This work led to an understanding of the important role of cell membranes in the RF range. The principal mechanism responsible for the β-dispersion is the accumulation of charges at membranes from extra-and intracellular fluids (Maxwell-Wagner effect). In addition, cell suspensions display the above discussed relaxations because of the presence of water and macromolecules in cell suspensions. Furthermore, they often display strong dispersions at low frequencies (α-dispersions). We summarize these cases in Table 2.

First we consider suspensions of cells or vesicles without macromolecules in the fluids inside and outside the cell. Then eqs. (9) and (13) are applicable. Assuming physiological conditions, these equations predict time constants corresponding to relaxation frequencies between 0.1 and 10 MHz for micron sized cells. Dielectric increments for volume fractions near 30% range in the thousands. Therefore suspensions display strong Maxwell-Wagner β-effects in the RF-range. Its magnitude is considerably greater than that of the β-effect of proteins. In addition the cell suspensions display a γ-effect because of water. The magnitude of the γ-effect corresponds

to the volume taken up by water.

Cells containing proteins (about 30% in the cytoplasm and 5-10% in the medium outside the cells) display the β- and γ-effects listed above. In addition the proteins add their own β-effect and cause the δ-effect due to their bound water. Relaxation frequencies of the β-dispersion of proteins are somewhat higher than those responsible for that of cells, but of much smaller magnitude. This results in a slight lifting of the high frequency tail of the β-dispersion. Cells with surface charges and/or dispersive membranes display α relaxation effects caused by the counter ion atmosphere and frequency dependent membrane properties. These effects are in addition to the other relaxations mentioned above.

Many cells possess membrane systems which extend from the cell membrane or the nuclear membrane into the cytoplasm. These structures can act like transmission line sections, causing frequency dependent contributions to the membrane properties. Characteristic frequencies are near 100 Hz for the tubular system of muscle. Thus apparent membrane properties change at low frequencies, causing an additional α-dispersion effect (Schwan 1954, Fatt 1964).

Organelles such as mitochondria and cell nuclei have dielectric responses identical to those of cells because of the presence of membranes separating electrolytes. Since they are small, their dispersions occur at higher frequencies and their magnitudes are smaller. Organelles inside cells contribute therefore to the tail of the β-dispersion of cell suspensions as may be predicted by eqs. (14) and (15) (Irimajiri *et al* 1979, Stoy *et al* 1982). The contributions of subcellular organelles diminish as the frequency decreases below the characteristic frequency of the cell suspensions. This is due to the shielding effect imposed by the cell membranes at lower frequencies, thereby reducing the field strength inside the cell. A detailed theory of a "multistratified shell" model has been applied to the case of organelles or nuclei inside the cell (Irimajiri *et al* 1979)

5. TECHNIQUES

Techniques for the determination of the dielectric properties of cells and tissues fall into two classes: a) Suspension techniques. b) Techniques for the study of single cells. Measurements a) integrate over many particles or cells and one obtains average electrical properties. In b), some of the ambiguities of suspension techniques are removed. However, this technique is usually more restrictive than a). Table 2 summarizes available technology, advantages and limitations.

5.1 Suspension techniques

5.1.3. Advantages and limitations of suspension techniques.

5.1.3.1. ***Electrode polarization.*** Low frequency limits to dielectric spectroscopy are imposed by electrode polarization (Schwan, 1963, 1968). The impedance of electrodes increases with decreasing frequency and, since in series with the suspension, adds increasingly to the measured impedance as the frequency is decreased. This is demonstrated by the following approximate equations (Schwan 1963)

$$C = C_s + \frac{G^2}{\omega^2 R^2 C_p} \tag{19}$$

$$R = R_s + R_p + R_s(R\omega C)^2 \tag{20}$$

R and C are measured resistance and capacitance, R_s and C_s are those of the sample, R_p and C_p are the components of the polarization impedance $Z_p = R_p - j/\omega C_p$. The equations are valid approximations if $R\omega C < 1$ and $R_p < R_s$[1]. Electrode artifacts contribute to measured capacitance values below about 3 KHz when cells are suspended in a physiological media ($\sigma \sim 10$ mS/cm)[2]. Large sample size, proper electrode treatment and methods to correct for the electrode effect can reduce this limit about tenfold. Electrode contributions to measured data decrease rapidly when the medium conductivity is reduced. They can be eliminated entirely by use of 4-electrode technology (Schwan, 1963, Schwan and Ferris, 1968). This technology uses different current and potential sensing electrodes. Care is required to exclude the potential sensing electrodes from currents and to avoid capacitive contributions which may mimic relaxation phenomena. No good commercial equipment is yet available for this purpose.

5.1.3.2. ***Resolution.*** Permittivity determinations are limited by the high dielectric loss tangent values $\tan \delta = \varkappa/\omega\varepsilon$ typical of cell suspensions at low frequencies (Schwan 1963). Resolution is determined by the principle $d\varkappa = \omega\, d\varepsilon$. For physiological media and typical dielectric constants of cell suspensions (ε near 2000), dielectric constants can be resolved to 10% at 1 KHz with equipment providing a resolution of 10^{-5} in impedance magnitude. Such resolution is only obtainable with few commercial products. Special laboratory devices providing resolutions as low as 10^{-6} and 10^{-7} have been reported (Schwan *et al* 1962, Schwan 1963, Hayakawa *et al,* 1975). Combination of 4-electrode technology with very high resolution equipment extends dielectric spectroscopy of biological solutions and suspensions to mHz frequencies.

5.1.3.3. ***Drift.*** Biological conductivities have a temperature coefficient of about 2%/°C. Conductivity resolutions near 10^{-5} require therefore temperature stabilization to 10^{-3} °C. This drift problem can be reduced by alternating measurements at various frequencies with those at a fixed reference frequency and to apply corrections using the data obtained at the reference frequency as time advances (Schwan 1963, Hayakawa *et al* 1975).

5.1.3.4. ***Mixture theories.*** Maxwell's mixture theory is not regorous since necessary assumptions include the need for low particle concentrations in order to exclude

[1] These conditions can be shown to apply if the polarization impedance components are smaller than those of the sample resistance (see Schwan, 1963). This is usually the case.

[2] Assuming a 10% error, equation (19) reduces to $f^2 C_p d\varepsilon = 3.10^{12}\sigma^2$. For example for a Pt electrode covered well with Pt-black C_p is about 1 mFarad/cm^2 at 1 KHz. Thus for $d = 5$cm, $\sigma = 0.01$ S/cm and $\varepsilon = 1000$ relative to free space, the frequency is $f = 2.4$ KHz.

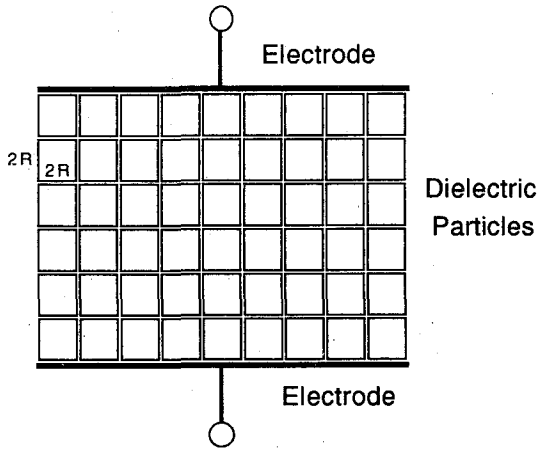

Fig. 3. A model of packed cubic dielectric particles. This model is used to prove the validity of the Maxwell's mixture theory even at high volume fraction.

the interference of other particles in the proximity. However, for low particle or cell concentrations the Maxwell theory is appropriate and agrees well with other mixture theories. How serious are the errors introduced by the simple Maxwell equation? We compare first the prediction of eq. (11), derived from Maxwell's equation (9) for the permittivity ε_0 of a concentrated cell suspension with that of the simple model indicated in Figure 3. This model assumes a densely packed number of cells of cubicle shape and size $2R$. N such cells are arranged in a unit volume between the electrodes. Thin membrane surfaces perpendicular to the electrodes do not contribute to the total capacitance and only the $2N$ membranes parallel to the electrodes need be considered. Therefore, $\varepsilon^0 = C_m/2N = RC_m$, since $2NR = 1$. This is identical with eq. (13b) for $p = 1$, indicating that the Maxwell prediction is good for high particle volume concentrations. Our own and other (Kell, personal communication), experimental experience support this conclusion. The validity of Maxwell's mixture equation has been also tested for the special case of nonconducting particles in a conducting medium and found to be excellent up to the highest possible concentrations (Cole *et al,* 1969). Nevertheless, it is uncertain if Maxwell's mixture equation is the best choice under different circumstances. More sophisticated theories avoid the assumption made by Maxwell. In particular, Hanai's equation (1968) has been successfully applied to suspensions of particles and cells and appears to be an extension of Bruggemann's equation to complex admittances (1935).

5.1.3.5. ***Membrane conductance.*** Determinations of membrane conductances with the suspension technique are difficult. The reason is apparent from equations (14) and (15): Typical G_m-values vary between 0.1 and 10 mS/cm² and therefore the second term in the denominator is small compared to 1 for typical cell size. Hence, the effect of the membrane conductance is usually small and difficult to extract from measured data. This problem can only be circumvented if the second term in the denominator is made large by choice of large cells and medium resistivity. However any increase in the

resistivity of the medium may adversely affect cell properties.

5.2 Single cell techniques

5.2.1. Microelectrode techniques

We discuss next the measurement of membrane capacitance and conductance of biological cells using methods which are more suitable to extract information about the membrane conductance. The membrane conductance is of greater interest than the membrane capacitance. The conductance is caused by channels and pores which regulate ion exchange and occupy only a small fraction of the membrane surface. Hence it is functionally more important than the capacitance which is mainly due to membrane bulk or the lipid layer.

Microelectrode Techniques

There are two types of microelectrodes, made of metal and of glass. Of particular importance is glass microelectrode with a very fine tip of less than 1 μm. These electrodes are inserted into the cell interior and transmembrane electrical properties such as the membrane potential are measured. Because of the very large tip impedance and various limitations thereof, i. e., limited frequency response and the propensity to be nonlinear, they are not suitable for the measurement of membrane admittances. In spite of these shortcomings, membrane capacitance and resistance can be measured using two such microelectrodes. With this technique, both electrodes are inserted into a cell. One of them is used for current injection and the other for voltage recording. The membrane impedance can be obtained as a transfer function between voltage and current: $Z=V/I$. This technique was successfully used for the measurement of the transmembrane impedance of skeletal muscle between 1Hz and 1KHz by Falk, *et al.* (1964).

Voltage Clamp Method

Originally, the voltage clamp technique was developed to investigate the current-voltage characteristics of squid axons (Cole, 1968). Using a negative feed-back control system and intra-cellular voltage sensing and current electrodes, the membrane potential of the nerve axon is clamped at a predetermined value, thus preventing the action potential from developing. Using this technique, a highly nonlinear current-voltage characteristics of nerve membranes was revealed (Hodgkin *et al.* 1952). From the tangent of these current diagrams, we can calculate the conductance of nerve membranes. In addition, using the capacitive transients, the total capacitance of the membrane can be determined. A schematic diagram of the voltage clamp circuit is illustrated in Fig.4.

Finally, with the advent of the patch clamp technique, it is possible to measure membrane properties of small spherical or ellipsoidal cells using a glass micropipet (Sakmann *et al.*, 1983). A glass micropipet is pressed against a small section of the cell wall with a gentle suction in order to establish tight seal with a very high resistance of more than 10^9 ohms. Application of a square pulse using the pipet and subsequent current recording permits determination of membrane current-voltage characteristics.

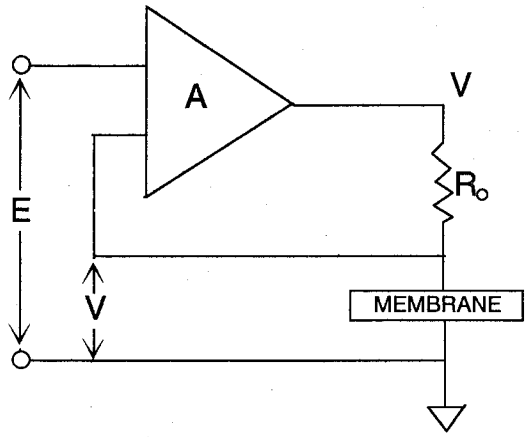

Fig. 4. Schematic diagram of the voltage clamp circuit. A is differential amplifier, R_o is access impedance, E is command potential and V is membrane potential.

Of particular interest of this technique is its ability to investigate membrane "patches" and to measure 'single channel currents'. Using a very small membrane patch which is tightly sealed at the tip of micropipet, spontaneous opening and closing of each channel can be recorded by monitoring small current pulses. Although channel opening and its duration are stochastic, the magnitude of single channel currents is usually of the order of 3-5 pA.

A variation of the patch clamp technique uses a glass micropipet tightly attached to the cell surface to measure the membrane admittance. Asami *et al* (1990), using sinusoidal voltage inputs, successfully measured membrane capacitance and conductance of small nearly spherical cells such as myeloma and HeLa cells between 1Hz and 1000Hz. In this frequency range they found frequency independent membrane properties $C_m \sim 0.8 \mu F/cm^2$ and $G_m < 100 \mu S/cm^2$.

5.2.2. Electro-rotation.

The rotation of particles and biological cells exposed to a rotating alternating field has been extensively investigated by Arnold and Zimmermann (Arnold 1988, Arnold and Zimmermann 1984, 1988, Zimmermann and Arnold 1983) and by Glaser and Fuhr (1986). It peaks at the characteristic frequencies of the β-dispersion as may be predicted by application of the simple cell model characterized by equations (10) to (14) (Schwan 1985). Fuhr *et al* carried out more extensive calculations analogous to the Pauly-Schwan theory memtioned above (1985, 1986). A rigorous discussion for arbitrary complex dielectric properties of particle and medium has been carried out by Sauer and Schloegl for cylinders and spheres (1985). The torque L which causes the particle to spin is given by $L = E\mu''$ with E field strength and μ'' the imaginary part of the induced dipole moment

$$\mu = \mu' + j\mu'' = \varepsilon_a R^3 E \frac{K_p - K_a}{K_p + 2K_a} = \varepsilon_a R^3 E u \tag{21}$$

R is the particle radius and the subscripts p and a indicate particle and outside medium. The K's are admittivities and ε_a is the permittivity of the medium. If the suspending medium is an electrolyte, ε_a is frequency independent up to more than 1 GHz. Thus the frequency dependence of the rotation speed is directly proportional to the quantity u''. The comparison of the suspension and cell rotation techniques and their advantages and disadvantages are shown in Table 2.

5.2.3. Levitation.

If a particle is exposed to a divergent electrical field, it experiences a force given by

$$F = \varepsilon_a u' \text{ grad } E^2 \tag{22}$$

where u' is now the real part of the ratio u defined in eq. (21). u' can be either positive or negative. Thus the force may be directed towards or away from the region of higher field intensity. This force may be used to balance gravitational forces, thus providing a null technique of potentially great accuracy. Stability criteria have been worked out and the technique demonstrated to be useful for the investigation of particles and biological cells (Kaler and Jones, 1990). The relevant theory is very similar to the one for the rotational response. However, the frequency response of the gradient force is similar to the dispersion effect discussed for dielectric spectroscopy. For example, in the case of uniform particles, the force changes with frequency as expressed by the dispersion equations discussed above in section 2. The magnitude of the dispersion is now

$$\Delta u' = \frac{3(\varepsilon_a \kappa_i - \varepsilon_i \kappa_a)}{(\kappa_i + 2\kappa_a)(\varepsilon_i + 2\varepsilon_a)} \tag{23}$$

and, hence, can be either positive or negative. The time constant is identical to the one obtained for rotation and dielectric spectroscopy. In the case of shell surrounded particles two dispersions result with the same time constants as in the cases of rotation and spectroscopy.

Rotation and levitation techniques have much in common with dielectric spectroscopy. The same equations are used to obtain cellular data from the particle admittivity K_p. In both cases u-transformations must be used as seen from the equations for torque L and force F. The difference is that u'' directly determines the rotational response and u' that of levitation, while the transformation on the left side of eq. (9) needs to be made in addition in order to relate suspension data to particle property. Thus the rotation and levitation techniques are one step more direct and not subject to the limitations of Maxwell's mixture eq. (9). However, it is not possible to extract from either u' or u'' alone the dielectric properties ε and κ of the particle observed. Additional information must be provided. This can be done for example by variation of the conductivity of the medium (Zimmermann and Arnold 1983). The suspension technique provides both components of u from observed ε and κ data. It is also faster, easier to apply, covers a much broader frequency range and commercial instrumenta-

Table 2. Advantages (indicated by +) and limitations (−)of dielectric spectroscopy (S) and electro-rotation (R). The rotation technique requires high field strength. This causes undue heating unless the medium's conductivity is reduced. The levitation and rotation technique share similar advantages and limitations.

ROTATION+DIELEC. SPECTROSCOPY		
	R	S
Single Cell Observation	+	−
Well explored		+
Frequency Range		+
Suspension Theory Problem	+	−
Several dispersions		+
Ease of Extraction of Electr. Param.		+
Temperature and Medium Limitations	−	+
Electrode Polarization Problem	+	−
α-dispersion	−	+
Equipment availability	−	+

tion is available. Information collected sofar with dielectric spectroscopy, electrorotation and levitation appear to be in agreement with regard to cellular parameters C_m, G_m and κ_i obtained from high frequency data. However the α-dispersion observed with charged particles and data obtained from electrorotation do not agree (Arnold *et al* 1987). It appears that rotating and non-rotating fields interact with the counterion atmosphere in a different manner.

Table 2 attempts to summarize the advantages and limitations of various techniques.

In addition to the techniques discussed above, yet another new method 'microspectroscopy' has been reported. The interested reader should be refered to the references (Washizu, 1989, 1990).

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